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Research Article

Green Synthesis and Characterization of Silver Nanoparticles Using *Nepeta deflersiana* Extract: A Novel Approach for Larvicidal Control of *Aedes aegypti*

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Abstract

Background: Dengue fever, transmitted by *Aedes aegypti* mosquitoes, is a major public health concern in Saudi Arabia. The emergence of insecticide resistance necessitates the development of eco-friendly alternatives.

Objective: This study aimed to synthesize and characterize silver nanoparticles (AgNPs) using *Nepeta deflersiana* (*N. deflersiana*) ethanol extract and evaluate their larvicidal activity against *Ae. aegypti* fourth-instar larvae.

Methods: *N. deflersiana* leaves were collected from Al Baha region, Saudi Arabia. Ethanol extract was prepared by maceration. AgNPs were biosynthesized using 1 mM silver nitrate and characterized by UV-Vis spectrophotometry, SEM, and FTIR. Phytochemical composition was analyzed by GC-MS. Larvicidal bioassays were conducted using the WHO immersion method with five concentrations for crude extract (500-1300 ppm) and AgNPs (0.1-0.9 ppm), Control groups included distilled water. Mortality was recorded after 24 hours, and data were analyzed by probit analysis.

Results: GC-MS analysis revealed 55 compounds, majorly 17-octadecynoic acid (14.50%), 3-pyridinecarboxaldehyde (9.45%), α-linolenic acid (8.44%), and phytol (7.63%). UV-Vis spectroscopy showed SPR peak at 420-440 nm. SEM revealed spherical nanoparticles (15-45 nm). FTIR confirmed involvement of -OH, C=O, and -NH groups. Crude extract exhibited LC_{50} of 791.482 ppm and LC_{90} of 1272.85 ppm. AgNPs showed dramatically enhanced activity with LC_{50} of 0.301 ppm and LC_{90} of 1.355 ppm based on probit analysis, representing a 2,630-fold increase in potency. Treated larvae exhibited cuticle melanization, siphon damage, setae destruction, and body shrinkage.

Conclusion: *N. deflersiana*-synthesized AgNPs show promising laboratory-based larvicidal activity against *Ae. aegypti*, offering a potential eco-friendly alternative for further investigation in dengue vector control. Further field evaluations, non-target toxicity assessments, and higher concentration testing are needed before practical application.

Keywords: *Nepeta deflersiana*; Silver Nanoparticles; Green Synthesis; *Aedes aegypti*; Larvicidal Activity; Dengue Vector; Saudi Arabia

Introduction

Dengue fever, transmitted by *Aedes aegypti* mosquitoes, poses a significant public health threat in the Kingdom of Saudi Arabia, particularly during mass gatherings such as Hajj and Umrah [1,2]. The first documented outbreak in Saudi Arabia occurred in Jeddah in 1994, and since then, the disease has spread to major cities including Makkah, Madinah, and Jazan [3,4]. The influx of millions of pilgrims from dengue-endemic regions worldwide creates unique public health challenges for authorities [5].

Traditional mosquito control strategies have relied heavily on chemical insecticides, including organophosphates (temephos, malathion) and pyrethroids [6]. However, the widespread and prolonged use of these chemicals has led to several critical issues: (1) development of insecticide resistance in mosquito populations; (2) environmental contamination of soil and water sources; (3) toxicity to non-target organisms including fish, bees, and beneficial aquatic insects; and (4) adverse effects on human health [7,8].

In response to these challenges, plant-based materials have emerged as promising alternatives for mosquito vector control [9,10]. Phytochemicals extracted from various plants have demonstrated mosquitocidal and repellent properties [6,11]. However, the relatively lower potency of crude plant extracts compared to synthetic insecticides has limited their practical application.

The emergence of nanotechnology offers a revolutionary approach to enhance the efficacy of plant-derived bioactive compounds [12]. Green synthesis of silver nanoparticles (AgNPs) using plant extracts combines the bioactivity of phytochemicals with the unique physicochemical properties of nanoparticles, including high surface area-to-volume ratio, enhanced cuticular penetration, and synergistic effects [13,14].

Nepeta deflersiana (Schweinf. ex Baker) is a perennial aromatic herb belonging to the Lamiaceae family, growing abundantly in the southern regions of Saudi Arabia [15]. Traditionally, the plant has been used for treating stomach ailments, pain, urinary tract infections, and burns. However, its potential for green synthesis of silver nanoparticles and larvicidal activity against *Ae. aegypti* has not been previously investigated.

Therefore, this study aimed to: (1) synthesize and characterize silver nanoparticles using *N. deflersiana* ethanol extract; (2) analyze the phytochemical composition of the extract using GC-MS; (3) evaluate the larvicidal activity of the crude extract against *Ae. aegypti* fourth-instar larvae; (4) evaluate the larvicidal activity of biosynthesized AgNPs; (5) examine morphological changes in treated larvae; and (6) compare the potency of AgNPs with crude extract.

Materials and Methods

Plant collection and authentication

Leaves of *Nepeta deflersiana* were collected from the Al Baha region, located in the southwestern part of Saudi Arabia. The plant samples were authenticated by Dr. Najeeb Al-Absi, Assistant Professor of Plant Taxonomy, Faculty of Sciences, Al-Baha University.

Figure 1: *Nepeta deflersiana* plant.

Preparation of plant extract

Fresh leaves of *N. deflersiana* were washed thoroughly with tap water followed by distilled water, then dried at room temperature (25 ± 2°C) for 7 days. Dried leaves were mechanically powdered using an electrical stainless steel blender. For extraction, 30 g of powder was saturated in 90 mL of ethanol (99.8% purity) for 72 hours on a mechanical shaker (Wise Shake) at 150 rpm. The mixture was filtered using Whatman No. 1 filter paper. The filtrate was concentrated using a rotary vacuum evaporator (Büchi R-300) at 40°C under reduced pressure. The crude extract was collected in dark glass containers and stored at 4°C until further use.

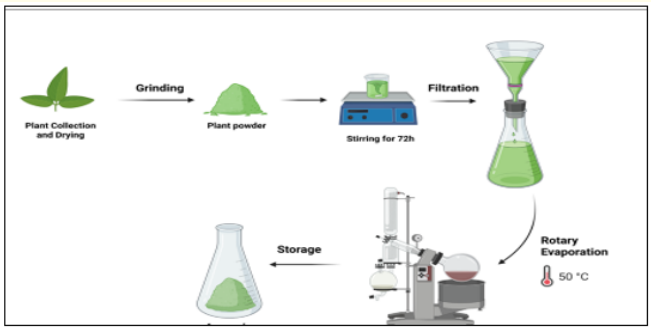

Figure 2: Schematic diagram step-by-step ethanol extraction and rotary evaporation of bioactive compounds from plant leaves extract.

GC-MS analysis of phytochemical composition

The phytochemical composition of *N. deflersiana* ethanol extract was analyzed using Gas Chromatography-Mass Spectrometry (GC-MS; Agilent 7890B GC coupled with 5977B MSD). Separation was performed on an HP-5MS capillary column (30 m × 0.25 mm × 0.25 µm). Helium was used as carrier gas at a flow rate of 1.0 mL/min. The oven temperature program was: initial temperature 60°C (hold 2 min), increased to 280°C at 10°C/min (hold 10 min). Injector temperature was 250°C, and ion source temperature was 230°C. Compounds were identified by comparing their mass spectra with the NIST (National Institute of Standards and Technology) library.

Biosynthesis of silver nanoparticles (AgNPs)

Silver nanoparticles were biosynthesized by adding 1 mM silver nitrate ($AgNO_3$, Sigma-Aldrich, purity ≥99.8%) solution to the plant extract at a ratio of 1:9 (extract: $AgNO_3$). The mixture was incubated at room temperature (25 ± 2°C) for 24 hours. Formation of AgNPs was confirmed by visual observation of color change from dark green to dark brown. The biosynthesized AgNPs were purified by centrifugation at 4000 rpm for 30 minutes, washed twice with distilled water, and dried at 40°C.

Characterization of silver nanoparticles

UV-Vis absorption spectra of the AgNP suspension were recorded using a UV-Vis/NIR spectrophotometer (Shimadzu UV-3600) in the range of 300-700 nm. Distilled water was used as a blank reference. The morphology and size of the biosynthesized AgNPs were examined using a Scanning Electron Microscope. The dried AgNP powder was placed on conductive carbon adhesive connectors and sputter-coated with gold before examination. Particle sizes were measured using ImageJ software. FTIR spectra of both crude extract and AgNPs were recorded using a Bruker Tensor 37 FTIR spectrometer in the range of 400-4000 cm^{-1}. Samples were prepared using the KBr pellet method.

Mosquito rearing

Aedes aegypti mosquitoes were reared at the Central Laboratory of Insecticide Bioassay, Research Unit of Dengue Fever and Vector Control, King Abdulaziz University, Jeddah. The colony was maintained at 27 ± 2°C, 75-85% relative humidity, and a 14:10 hour light:dark photoperiod. Larvae were fed a mixture of yeast, dried milk, and bread (1:1:1 ratio). Adult mosquitoes were provided a 10% sugar solution, and females were blood-fed on pigeons for egg production. The Dengue Fever Research Unit is a specialized bio-based unit within the Bio-Evaluation Department at King Abdulaziz University. Pigeon blood-feeding is a standard practice for mosquito colony maintenance and was conducted in accordance with institutional guidelines with minimal discomfort to the animals.

Larvicidal bioassays

Crude extract bioassay

The larvicidal activity of *N. deflersiana* crude extract was evaluated against fourth-instar *Ae. aegypti* larvae using the standard WHO immersion method [25]. Five concentrations were tested: 500, 700, 900, 1100, and 1300 ppm. Each concentration was tested with five replicates, each containing 20 larvae in 100 mL of distilled water containing 0.5% Triton X-100 as a surfactant. Control groups received distilled water with 0.5% Triton X-100 without plant extract. Mortality was recorded after 24 hours of exposure. Larvae were considered dead if they did not respond to touch with a dissection needle.

AgNP bioassay

The larvicidal activity of biosynthesized AgNPs was evaluated using five concentrations: 0.1, 0.3, 0.5, 0.7, and 0.9 ppm. Each concentration was tested with five replicates, each containing 20 fourth-instar larvae in 100 mL of distilled water. Control groups received distilled water only. Mortality was recorded after 24 hours.

Figure 3: Steps of experimental larval mortality induced by plant extract and its NPs against *Aedes aegypti*.

Correction of mortality

Mortality percentages were corrected using Abbott's formula when control mortality exceeded 5%:

$$\text{Corrected Mortality \%} = \frac{(\text{Treatment Mortality \% - Control Mortality \%})}{(100 - \text{Control Mortality \%})} \times 100$$

Morphological observations

Dead larvae were collected and examined for morphological abnormalities using a Stereo Dissection Microscope (Leica EZ4D) equipped with a digital camera. Control larvae were examined for comparison. Photographs were taken at various magnifications.

Statistical analysis

Mortality data were analyzed using probit analysis according to Finney [26] to determine LC_{50} (median lethal concentration) and LC_{90} (90% lethal concentration) values with their 95% confidence limits, regression equations, slope values, chi-square (χ^2), and correlation coefficients (r). The activation ratio (enhancement factor) was calculated as:

$$\text{Activation Ratio} = \frac{LC_{50}\ (\text{crude extract})}{LC_{50}\ (\text{AgNPs})}$$

Results

Phytochemical composition by GC-MS analysis

The GC-MS analysis of *Nepeta deflersiana extract revealed a complex phytochemical profile consisting of 55 distinct compou*nds with varying retention times and peak areas (Figure 4). The chromatogram showed a series of peaks ranging from 6.60 to 44.99 minutes, with the most abundant compounds eluting between 13 and 31 minutes. The major compound identified in this extract was 17-octadecynoic acid, which appeared at multiple retention times (15.20, 17.28, 18.13, 21.29, and 24.91 minutes) with a cumulative area percentage of approximately 18.5%, the highest being 14.50% at 18.13 minutes (Table S1). Other predominant compounds included 3-pyridinecarboxaldehyde with a peak area of 9.45% at 38.04 minutes, benzene-1-methoxy-4-nitro- (8.89% at 13.39 minutes), cyclopentanecarboxylic acid-2-acetyl-5-

methyl- (8.89% at 14.57 minutes), and 9,12,15-octadecatrienoic acid (α-linolenic acid) with 8.44% at 30.43 minutes. Additionally, phytol (2-hexadecen-1-ol,3,7,11,15-tetramethyl-) was identified at 29.95 minutes with a peak area of 7.63%, while (4aS,7S,7aR)-4,7-dimethyl-5,6,7,7a-tetrahydrocyclopenta[c]pyran-1(4aH)-one contributed 6.93% at 13.25 minutes. Minor compounds included linalool (0.14%), caryophyllene oxide (0.31%), and various fatty acid derivatives. The presence of these terpenoids, fatty acids, and phenolic compounds suggests that *N. deflersiana possesses diverse bioactive constituents potentially responsible for its larvicidal properties.*

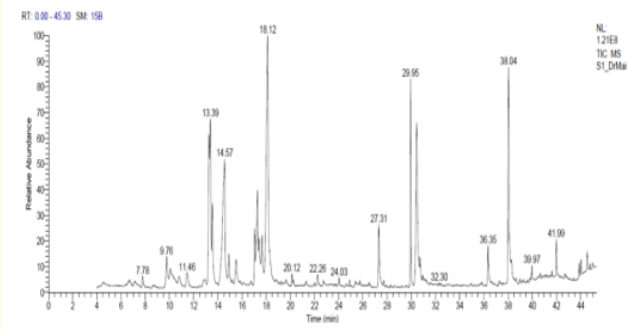

Figure 4: GC-MS chromatogram of *Nepeta deflersiana* (P1) ethanol extract showing the separation of phytochemical compounds.

RT (min)	Compound Name	Area (%)	Molecular Formula	MW	CAS #
6.60	2,2-Dimethyl-3-vinyl-bicyclo [2.2.1]h eptane	0.16	C11H18	150	115948 -98-6
6.70	2-Furanmethanol,5-ethenyltetrahydro-à, à,5-trimethyl-, cis-	0.28	C10H18O2	170	5989-3 3-3
7.08	Linalool	0.14	C10H18O	154	78-70-6
7.16	1-PENTANOL,5-CYCLOPROPYLIDEN-	0.25	C8H14O	126	162377 -97-1
7.77	13-OXABICYCLO [10.1.0] TRIDECA NE	0.48	C12H22O	182	286-99 -7
9.76	13-OXABICYCLO [10.1.0]TRIDECA NE	1.41	C12H22O	182	286-99 -7
10.07	5H-1-PYRINDINE,6,7-DIHYDRO-7-METHYL-	0.48	C9H11N	133	54664-5 7-2
10.81	1-Undecyne	0.64	C11H20	152	2243-9 8-3
11.44	3,4-Octadiene, 7-methyl-	1.00	C9H16	124	37050-0 5-8
12.92	2-Cyclohexen-1-one,3-methyl-6-(1-methylethyl)-	0.75	C10H16O	152	89-81-6
13.25	(4aS,7S,7aR)-4,7-Dimethyl-5,6,7,7atetrahydrocyclopenta[c]pyran-1(4aH) -one	6.93	C10H14O2	166	21651-6 2-7
13.39	BENZENE,1-METHOXY-4-NITRO-	8.89	C7H7NO3	153	100-17 -4
13.55	2-Octyne, 1,1-diethoxy	2.55	C12H22O2	198	16387-5 5-6
14.57	Cyclopentanecarboxylic acid,2-acetyl-5-methyl-	8.89	C9H14O3	170	528-25 -6
14.93	1-Undecyne	1.21	C11H20	152	2243-9 8-3
15.20	17-Octadecynoic acid	0.14	C18H32O2	280	34450-1 8-5
15.50	E-7-Tetradecenol	1.25	C14H28O	212	37011-9 5-3
16.73	,8,11,14-EICOSATETRAENOIC ACID, METHYL ESTER, (ALL-Z)-	0.16	C21H34O2	318	2566-8 9-4
17.04	(2-HYDROXYTRICYCLO[4.3.0.0~3,8~] NON-2-YL) METHANAMINIU M CHLORIDE	1.80	C10H18ClNO	203	102861 -20-1
17.28	17-Octadecynoic acid	3.50	C18H32O2	280	34450-1 8-5
17.42	(4aS,7S,7aR)-4,7-Dimethyl-2,4a,5,6,7,7a-hexa-hydro-1H-cyclopenta[c]pyri din-1-one	0.84	C10H15NO	165	115421 -73-3

17.66	TRICYCLO [4.3.1.1(3,8)] UNDECAN -1-AMINE	1.56	C11H19N	165	31083-6 1-1
18.13	17-Octadecynoic acid	14.50	C18H32O2	280	34450-1 8-5
18.30	9,12-Octadecadienoyl chloride, (Z,Z)-	0.21	C18H31ClO	298	7459-3 3-8
18.39	HEXADECADIENOIC ACID, METHYL ESTER	0.09	C17H30O2	266	29961-5 4-4
19.28	Longipinene epoxide	0.12	C15H24O	220	142792 -93-6
19.63	Cholestan-3-ol, 2-methylene-, (3á,5à)-	0.30	C28H48O	400	22599-9 6-8
20.12	3-Buten-2-one,4-(2,2,6,7-tetramethyl-7-azabicyclo [4 .1.0] heptan-1-yl)-	0.50	C14H23NO	221	102635 -60-9
20.25	-(2,2-Dimethyl-6-methylenecyclohe xyl) butanal	0.36	C13H22O	194	95452-1 3-4
21.29	17-Octadecynoic acid	0.25	C18H32O2	280	34450-1 8-5
21.98	4-(2,2-Dimethyl-6-methylenecyclohe xyl) butanal	0.14	C13H22O	194	95452-1 3-4
22.27	5,8,11,14-Eicosatetraenoic acid, methyl ester, (all-Z)-	0.59	C21H34O2	318	2566-8 9-4
22.74	6-Octadecynenitrile	0.32	C18H31N	261	56600-1 9-2
24.03	1,2-Epoxy-5,9-cyclododecadiene	0.39	C12H18O	178	943-93 -1
24.91	17-Octadecynoic acid	0.24	C18H32O2	280	34450-1 8-5
25.38	Cholestan-3-ol, 2-methylene-, (3á,5à)-	0.19	C28H48O	400	22599-9 6-8
25.74	9,12-Octadecadienoyl chloride, (Z,Z)-	0.19	C18H31ClO	298	7459-3 3-8
26.51	HEXADECADIENOIC ACID, METHYL ESTER	0.16	C17H30O2	266	29961-5 4-4
27.31	9-OCTADECENOIC ACID (Z)-	2.80	C18H34O2	282	112-80 -1
27.72	Hexadecanoic acid, ethyl ester	0.22	C18H36O2	284	628-97 -7
28.27	1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl ester	0.12	C21H38O2	322	56687-6 8-4
29.95	2-HEXADECEN-1-OL,3,7,11,15-TETRAMETHYL-, [R-[R*,R*-(E)]]-	7.63	C20H40O	296	150-86 -7
30.43	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	8.44	C18H30O2	278	463-40 -1
30.72	8,11,14-Eicosatrienoic acid, (Z,Z,Z)-	0.52	C20H34O2	306	1783-8 4-2
30.95	9-OCTADECENOIC ACID (Z)-	0.28	C18H34O2	282	112-80 -1
31.10	8,11,14-Eicosatrienoic acid, (Z,Z,Z)-	0.19	C20H34O2	306	1783-8 4-2
31.25	6,9,12-OCTADECATRIENOIC ACID, METHYL ESTER	0.13	C19H32O2	292	2676-4 1-7
32.30	CHOLESTAN-3-OL, 2-METHYLENE-, (3á,5à)-	0.12	C28H48O	400	22599-9 6-8
35.82	6,9,12-OCTADECATRIENOIC ACID, METHYL ESTER	0.20	C19H32O2	292	2676-4 1-7
36.34	2(3H)-Benzofuranone, hexahydro-4,4,7a-tri-methyl-	1.76	C11H18O2	182	16778-2 7-1
37.26	HEXADECADIENOIC ACID,METHYL ESTER	0.25	C17H30O2	266	29961-5 4-4
38.04	3-PYRIDINECARBOXALDEHYDE	9.45	C8H10N2OS	182	91540-4 2-0
38.29	Caryophyllene oxide	0.31	C15H24O	220	1139-3 0-6
38.66	Aromadendrene oxide-(2)	0.20	C15H24O	220	NA
38.97	6,9,12-OCTADECATRIENOIC ACID, METHYL ESTER	0.20	C19H32O2	292	2676-4 1-7

39.22	HI-OLEIC SAFFLOWER OIL	0.14	C21H22O11	450	8001-2 3-8
39.82	6,9,12-OCTADECATRIENOIC ACID, METHYL ESTER	0.14	C19H32O2	292	2676-4 1-7
39.97	1,25-Dihydroxyvitamin D3, TMS derivative	0.55	C30H52O3Si	488	55759-9 4-9
40.67	6,9,12-OCTADECATRIENOIC ACID, METHYL ESTER	0.17	C19H32O2	292	2676-4 1-7
41.63	CYCLODODECANEPENTANOIC ACID, 1-NITRO-á,2-DIOXO-, PHENYLMETHYL ESTER	0.23	C24H33NO6	431	86572-3 3-0
41.99	CHOLAN-24-OIC ACID,3,7,12-TRIHYDROXY-,(3à,5á,7à,12à)-	1.43	C24H40O5	408	81-25-4
42.69	Cholestan-3-ol, 2-methylene-, (3á,5à)-	0.21	C28H48O	400	22599-9 6-8
43.88	1,25-Dihydroxyvitamin D3, TMS derivative	0.60	C30H52O3Si	488	55759-9 4-9
44.03	1-Heptatriacotanol	0.63	C37H76O	536	105794 -58-9
44.54	13-HEPTADECYN-1-OL	0.79	C17H32O	252	56554-7 7-9
44.78	HI-OLEIC SAFFLOWER OIL	0.17	C21H22O11	450	8001-2 3-8
44.99	6,9,12,15-Docosatetraenoic acid, methyl ester	0.27	C23H38O2	346	17364-3 4-0

Table S1: Phytochemical composition of *Nepeta deflersiana* (P1) ethanol extract identified by GC-MS analysis.

Biosynthesis of silver nanoparticles

The biosynthesis of AgNPs was initially confirmed by visual observation of color change. Upon addition of 1 mM $AgNO_3$ to the *N. deflersiana extract, the color changed from dark green to a distinctive dark brown within 30 minutes. The color intensity increased progressively over 24 hours, indicating continued nanoparticle formation. No color change was observed in the control (extract without* $AgNO_3$) (Figure 5).

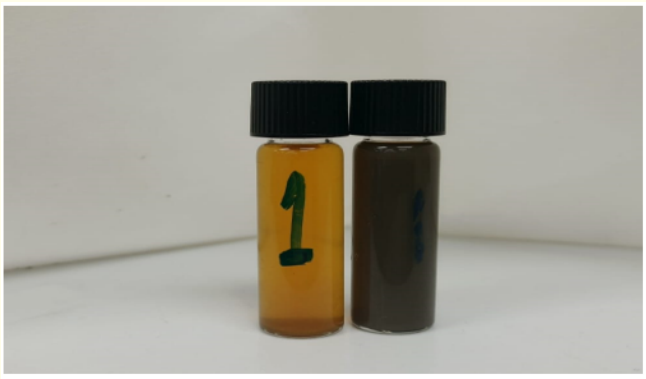

Figure 5: Biosynthesis of silver nanoparticles. Before Biosynthesis and after Biosynthesis. (A)- Before Biosynthesis, (B) -After Biosynthesis.

UV-Vis spectrophotometry analysis

The UV-Vis absorption spectrum of *N. deflersiana*-AgNPs showed a sharp surface plasmon resonance (SPR) peak at 420-440 nm with high absorbance intensity. The peak was relatively symmetrical, indicating uniform particle size distribution. The control (plant extract without $AgNO_3$) showed no absorption peak in the 400-500 nm region.

Scanning electron microscopy (SEM) analysis

SEM analysis revealed that *N. deflersiana*-AgNPs were predominantly spherical in morphology with smooth surfaces and well-defined boundaries. The particles ranged in size from approximately 15 to 45 nm, with the majority falling within the 20-35 nm range. Some minor aggregation was observed, which is common during the drying process required for SEM sample preparation (Figure 6).

FTIR spectroscopy analysis

FTIR spectra of the crude extract and AgNPs showed characteristic peaks corresponding to various functional groups. After AgNP synthesis, shifts in peak positions were observed, confirming the involvement of these functional groups in the reduction and capping processes (Figure 7).

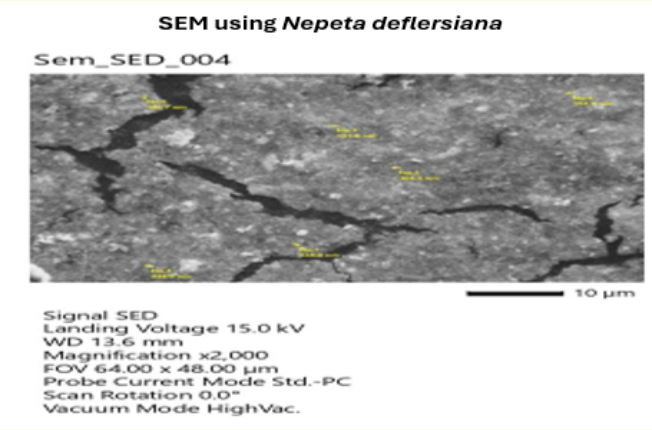

Figure 6: Scanning electron microscopy (SEM) image of *Nepeta deflersiana*-AgNPs showing spherical morphology and size distribution.

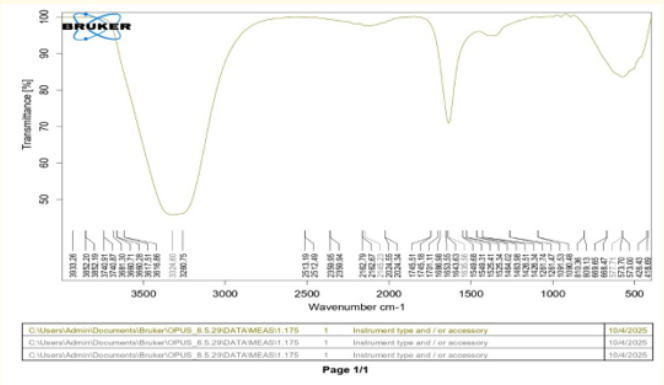

Figure 7: FTIR spectra of *Nepeta deflersiana* crude extract and its biosynthesized AgNPs showing functional group shifts.

Larvicidal activity of crude extract

The larvicidal activity of all ethanol plant extract against fourth-instar larvae of *Aedes aegypti was evaluated using the standard WHO immersion method, with mortality recorded after 24 hours of continuous exposure. Five concentrations (500, 700, 900, 1100, and 1300 ppm) were tested, each with 100 larvae (20 larvae per replicate × 5 replicates).*

Toxicity of *Nepeta deflersiana* extract (P1)

The larvicidal activity of *Nepeta deflersiana ethanol extract demonstrated concentration-dependent larvicidal activity, with mortality rates increasing progressively as the concentration increased. At the lowest tested concentration of 500 ppm, the extract caused 28% larval mortality. When the concentration was increased to 700 ppm, mortality rose to 62%, representing more than a two-fold increase (*Appendix 1). Further concentration increases produced progressively higher mortality rates: at 900 ppm, mortality reached 78%; at 1100 ppm, 88%; and at the highest concentration of 1300 ppm, the extract achieved 92% mortality. The control group (exposed to distilled water containing 0.5% Triton X-100 without plant extract) showed no mortality throughout the experiment, confirming that the observed effects were solely due to the bioactive compounds present in the plant extract. Probit analysis of the concentration-mortality data yielded a regression equation with a slope of 4.8699 ± 0.4558, indicating a steep concentration-response relationship (Figure 8). The chi-square value (1.3566) was less than the tabulated value (7.8), and the p-value (0.7157) was greater than 0.05, confirming the goodness-of-fit of the probit model. The correlation coefficient (r = 0.9943) exceeded the tabulated value (0.878), indicating a strong positive relationship between log-concentration and probit mortality. The LC_{50} value for *N. deflersiana extract was calculated as 791.482 ppm, with 95% confidence* limits ranging from 752.481 to 829.764 ppm. The LC_{90} value was determined to be 1272.85 ppm. These results demonstrate that *N. deflersiana possesses significant larvicidal properties against Ae. aegypti larvae.*

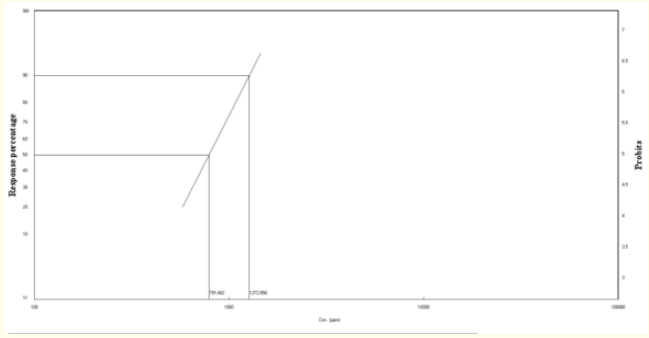

Figure 8: Probit regression line for *Nepeta deflersiana* crude extract (P1).

Larvicidal activity of biosynthesized silver nanoparticles (AgNPs)

The larvicidal activity of biosynthesized silver nanoparticles from all plant extracts were evaluated against fourth-instar *Ae. aegypti larvae using five concentrations (0.1, 0.3, 0.5, 0.7, and 0.9 ppm), each tested with 100 larvae (20 larvae × 5 replicates).*

Toxicity of *Nepeta deflersiana*-AgNPs (P1 Nano)

The results of larvicidal activity of biosynthesized silver nanoparticles from *Nepeta deflersiana (N. deflersiana*-AgNPs) demonstrated significantly enhanced larvicidal activity compared to the crude extract, with effective concentrations reduced by approximately three orders of magnitude. At the lowest concentration of 0.1 ppm, *N. deflersiana*-AgNPs caused 32% larval mortality. When the concentration was increased to 0.3 ppm, mortality rose to 36%; at 0.5 ppm, 46%; at 0.7 ppm, 56%; and at the highest concentration of 0.9 ppm, the AgNPs achieved 58% mortality (Figure 9). The control group showed no mortality throughout the experiment. Probit analysis of the concentration-mortality data yielded a regression equation with a slope of 0.7428 ± 0.1722. The chi-square value (2.7578) was less than the tabulated value (7.8), and the p-value (0.4305) was greater than 0.05, confirming the goodness-of-fit of the probit model. The correlation coefficient (r = 0.9343) exceeded the tabulated value (0.878), indicating a strong positive correlation. The LC_{50} value for *N. deflersiana*- AgNPs was calculated as 0.301 ppm, with 95% confidence limits ranging from 0.255 to 0.347 ppm. The LC_{90} value was determined to be 1.355 ppm. Compared to the crude extract (LC_{50} = 791.482 ppm), the AgNPs showed a remarkable 2,629-fold increase in potency (Resistance Ratio = 2629.508), demonstrating the dramatic enhancement effect of nanoparticle formulation.

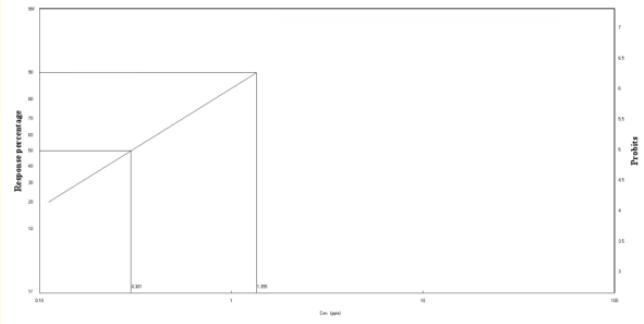

Figure 9: Probit regression line for *Nepeta deflersiana*-AgNPs (P1 Nano).

Enhancement effect of silver nanoparticles on larvicidal activity

Activation ratio of plant extracts after AgNPs synthesis

The biosynthesis of silver nanoparticles (AgNPs) using the five plant extracts resulted in a dramatic enhancement of larvicidal activity against *Aedes aegypti fourth-instar larvae. This enhancement was quantified by calculating the activation ratio, defined as the LC_{50} of the crude plant extract divided by the LC_{50} of the corresponding* AgNPs. A higher activation ratio indicates greater enhancement of activity following nanoparticle synthesis.

For *Nepeta deflersiana*, the crude extract exhibited an LC_{50} of 791.482 ppm, while the biosynthesized AgNPs showed an LC_{50} of 0.301 ppm, yielding an activation ratio of 2,629.5. This means that *N. deflersiana*-AgNPs were approximately 2,630 times more potent than the crude extract. The dramatic enhancement reflects the combined effects of the nanoparticle formulation, which provides increased surface area for interaction with larval tissues, enhanced penetration through the larval cuticle, and the synergistic action of phytochemical capping agents on the nanoparticle surface.

	LC50	Lower limit	Upper limit	Resistance Ratio (RR)
P1 nano	0.301	0.255	0.347	1
P 1 extract	791.482	752.481	829.764	2629.508

Table 1: Activation ratios of crude plant extracts P1 after biosynthesis of silver nanoparticles against *Aedes aegypti fourth-instar larvae*.

Morphological observations of treated larvae

Control larvae exhibited normal morphology with a translucent, pale cream-colored body, distinct head capsule, intact lateral and caudal setae, and a straight, elongated siphon. They showed active, coordinated swimming behavior. Larvae treated with crude extract (900-1300 ppm) exhibited progressive cuticle darkening (melanization) starting from the head capsule and siphon tip, spreading to thoracic and abdominal segments. At higher concentrations, the cuticle became completely blackened, wrinkled, and partially disintegrated. The siphon showed progressive darkening, wrinkling, and collapse. Lateral and caudal setae became clumped, bent, or completely lost. Body length was reduced by 20-40%. Treated larvae exhibited erratic, uncoordinated swimming, spiraling, and loss of normal orientation (Figure 10).

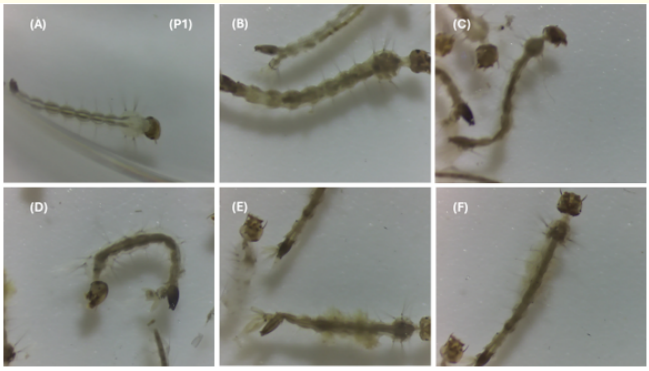

Figure 10: Morphological abnormalities in extract-treated larvae (P1).

AgNP-Treated larvae

Larvae treated with AgNPs (0.5-0.9 ppm) showed more severe abnormalities. Visible cuticle darkening occurred within 1-2 hours (compared to several hours for extracts). The siphon collapsed completely, becoming flattened or twisted, and in many larvae, the siphon became completely detached. Setae were completely destroyed, appearing dissolved or broken off at their bases. Body length was reduced by 50-70%. The head capsule softened and collapsed, with mandibles and mouthparts becoming detached. Internal tissue damage was visible through the partially translucent cuticle. Behaviorally, within 30-60 minutes of exposure, larvae exhibited violent, uncoordinated twitching and spiraling, followed by rapid immobilization and death within 6-12 hours (Figure 11).

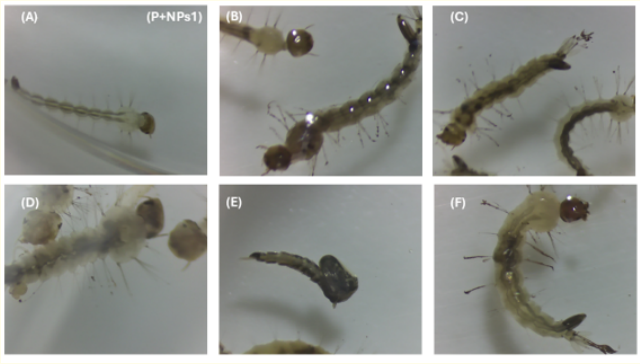

Figure 11: Morphological abnormalities in AgNP-treated larvae (P1 Nano).

Discussion

The GC-MS analysis revealed that *N. deflersiana contains a diverse array of bioactive compounds, including 17-octadecynoic acid (14.50%), α-linolenic acid (8.44%), and phytol (7.63%). These findings are consistent with previous studies on Nepeta species, which are known to produce various terpenoids and fatty acids with insecticidal properties* [16,17]. The presence of α-linolenic acid, a polyunsaturated fatty acid with three double bonds, is particularly significant. This compound is susceptible to autoxidation, generating reactive oxygen species (ROS) that cause cellular damage in target insects [13]. Similarly, phytol, a diterpene alcohol, has been documented for its ability to disrupt the endocrine system of insects and interfere with molting and metamorphosis [18]. The successful biosynthesis of AgNPs was confirmed by the color change from dark green to dark brown, which is characteristic of the surface plasmon resonance (SPR) phenomenon [12,14]. The SPR peak at 420-440 nm is typical for spherical silver nanoparticles, consistent with previous reports [13,19]. SEM analysis revealed spherical nanoparticles ranging from 15-45 nm, which is comparable to AgNPs synthesized using other plant extracts [14,20]. The FTIR analysis confirmed the involvement of hydroxyl (-OH), carbonyl (C=O), and amine (-NH) groups in the reduction and capping processes. The shifts in peak positions after AgNP synthesis indicate coordination of these functional groups with the nanoparticle surface [12,21].

The crude extract exhibited concentration-dependent larvicidal activity with an LC_{50} of 791.482 ppm. This potency is comparable to other Lamiaceae plant extracts reported in the literature [22,23]. The steep slope (4.8699) indicates a strong concentration-response relationship, meaning that small increases in concentration produce large increases in mortality. The most significant finding was the dramatic enhancement of larvicidal activity following AgNP synthesis. The LC_{50} decreased from 791.482 ppm to 0.301 ppm, representing a 2,630-fold increase in potency (99.96% reduction). This enhancement is comparable to or greater than previous reports. Morejón., *et al*. [12] reported a 6,600-fold enhancement for *Ambrosia arborescens*-AgNPs, while Raguvaran., *et al*. [13] reported 1,500- to 2,000-fold enhancement for *Aerva lanata*-AgNPs.

The morphological abnormalities observed in treated larvae, particularly siphon detachment and cuticle disintegration, suggest

that AgNPs cause rapid and extensive tissue damage leading to death by asphyxiation and desiccation [19]. The rapid onset of toxicity (30-60 minutes) indicates that AgNPs act more quickly than crude extracts, which required several hours to produce visible effects. These findings are consistent with previous reports that AgNP-treated larvae exhibit severe cuticular damage, muscle tissue degeneration, and fat body lysis [13,22]. The complete detachment of the siphon observed in many AgNP-treated larvae is a novel finding that has significant implications for the mechanism of action, as it immediately impairs the larva's ability to respire.

Conclusion

This study successfully demonstrated the green synthesis of silver nanoparticles (AgNPs) using *Nepeta deflersiana ethanol extract, which contained bioactive compounds including 17-octadecynoic acid (14.50%), α-linolenic acid (8.44%), and phytol (7.63%) that served as effective reducing and capping agents. The biosynthesized* AgNPs were spherical (15-45 nm) with an SPR peak at 420-440 nm. The crude extract exhibited an LC_{50} of 791.482 ppm, while *N. deflersiana*-AgNPs showed dramatically enhanced activity with an LC_{50} of 0.301 ppm, representing a 2,630-fold increase in potency (99.96% reduction). AgNP-treated larvae exhibited severe morphological abnormalities including rapid cuticle darkening, complete siphon detachment, extensive body shrinkage (50-70%), and death within 6-12 hours. The current study provides a strong foundation for future research; however, findings are based on laboratory conditions. Further studies including field evaluations, non-target toxicity assessments, enzyme mechanism investigations, and testing of higher AgNP concentrations are necessary before practical application. Commercial development and policy implementation should be considered as future research directions rather than immediate conclusions. Therefore, *N. deflersiana*-synthesized AgNPs *show promising laboratory-based larvicidal activity and represent a potential eco-friendly alternative that warrants further investigation.*

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Conflict of Interest Statement

The author declares no conflict of interest.

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